

REMARKS

It is respectfully submitted that the amendments submitted herewith function only to present a substitute specification containing proper margins and to insert the sequence listing and appropriate sequence identifiers into the text of the present application to comply with 37 C.F.R. §1.821 to 1.825. These amendments are made without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

It is respectfully submitted that the Sequence Listing conforms to the requirements of 37 C.F.R. §1.823(b). The Statements required by 37 C.F.R. §1.821(f) and (g) are set forth below.

Pursuant to 37 C.F.R. §1.821 (g), the undersigned hereby states that this submission, filed in accordance with 37 C.F.R. §1.821 (g), does not contain new matter.

Pursuant to 37 C.F.R. §1.821 (f), the undersigned hereby states that the content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821 (c) and (e), respectively, are the same.

The undersigned attorney of record hereby affirms and confirms that no new subject matter is added by the attached Substitute Specification and should now place this application in condition for allowance.

CONCLUSION

In view of the amendments, remarks and enclosures herein, it is respectfully submitted that the application now complies with all requirements set forth in the Notice. Accordingly, reconsideration and withdrawal of the Notice is respectfully requested.

Respectfully submitted,

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Enclosures: Paper and Diskette copies of Sequence Listing
Substitute Specification
Copy of Notice to Response To Notice To File Corrected Application Papers
Return receipt postcard

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Page 65, line 1:

Cassette 1- Translation initiation signal and signal peptide

In order to achieve correct translation initiation and secretion from mammalian cells, the following sequence is used (SEQ ID NO 16):

AagcttCCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGT
GTCCACTCC (SEQ ID NO: 38)

This contains a convenient *Hind*III restriction site for cloning into expression vectors (lower case), the consensus translation initiation signal for mammalian cells (ANNATGPu) and the coding sequence for a signal peptide sequence from an immunoglobulin gene.

Page 70, line 6:

The flexible linker, used to join the extracellular domain of B7.1 and the ScFv, was constructed by annealing two homologous oligonucleotides with engineered 5' Sma I and 3' Spe I sites - using oligonucleotides

Upper (SEQ ID NO: 6)

5' GGG GGT GGT GGG AGC GGT GGT GGC GGC AGT GGC GGC GGC GGA A 3'

and lower (SEQ ID NO: 16)

5' CTA GTT CCG CCG CCG CCA CTG CCG CCA CCA CCG CTC CCA CCA CCC CC 3'

The linker is cloned into pBluescript (Stratagene) via Sma I and Spe I to produce pLINK. The signal peptide (sp) and extracellular domain of murine B7.1 were amplified by PCR from pLK444-mB7.1 (supplied by R. Germain NIH, USA) via primers that introduce 5' EcoRI and 3' Sma I sites - primers forward

Page 70, line 18:

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5' C TCG AAT TCC ACC ATG GCT TGC AAT TGT CAG TTG ATG C 3'

reverse (SEQ ID NO: 18)

5' CTC CCC GGG CTT GCT ATC AGG AGG GTC TTC 3'

The B7.1 PCR product was cloned into pLINK via Eco RI and Sma I to form pBS/B7Link.

The V_H and V_L of the 5T4 specific ScFv was amplified via primers -

forward primer (SEQ ID NO: 19)

5' CTC ACT AGT GAG GTC CAG CTT CAG CAG TC 3'

reverse primer (SEQ ID NO: 20)

5' CTC GCG GCC GCT TAC CGT TTG ATT TCC AGC TTG GTG CCT CCA CC 3'

that introduce 5' Spe I and 3' Not I sites from pHEN1-5T4 ScFv. PBS/B7Link was digested with Spe I and Not I and ligated with the ScFv to form OBM 233 consisting of the sequence shown as SEQ ID No. 11: B7 Link ScFv sequence (Figure 5).

Page 73, line 5:

The sequence encoding a translation initiation sequence and the human immunoglobulin kappa light chain signal peptide is synthesized as two complementary single stranded oligonucleotides

which when annealed also contain an internal *Xho I* site at the 5' end and in addition leave a *Xba I* compatible 5' overhang and a *Pst I* compatible 3' overhang

ctagactcgagCCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA
GCT ACA GGT GTC CAC TCC GAG GTC CAG ctgca (SEQ ID NO: 21)

and

g CTG GAC CTC GGA GTG GAC ACC TGT AGC TGT TGC TAC CAA GAA GAG GAT
GAT ACA GCT CCA TCC CAT GGTGGctcgagt (SEQ ID NO: 22)

This is then cloned into pBluescript II (Stratagene) restricted with *Xba I* and *Pst I* to create pBSII/Leader.

Page 72, line 21:

The 5T4 ScFv is amplified by PCR from pHEN1 using oligonucleotides which incorporate a *Pst I* site at the 5' end of the product and a *Hind III* at the 3' end

GTC CAG CTG CAG CAG TCT GG (SEQ ID NO: 23)

and

CG TTT GAT TTC AAG CTT GGT GC (SEQ ID NO: 24)

This is then restricted with those enzymes and inserted into pBSII/Leader restricted with the same enzymes, creating pBSII/Leader/ScFv.

Page 74, line 1:

The HIgG 1 constant region is amplified by PCR from the cloned gene using oligonucleotides which incorporate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gcgc AAG CTT gaa atc aaa cgg GCC TCC ACC AAG GGC CCA (SEQ ID NO: 25)

and

gcgc ctcgag TCA TTT ACC CGG AGA CAG GG (SEQ ID NO: 26)

This is then restricted with those enzymes and inserted into pBSII/Leader/ScFv restricted with the same enzymes, creating pBSII/Leader/ScFv/HG1. The sequence for this construct is shown in the Figure 4 (SEQ ID No 10).

Page 74, line 29:

This fusion construct is made by amplifying the human IgE1 constant heavy region by PCR cDNA derived from human B-cells RNA by RT and subsequently using oligonucleotides which incorporate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gcgc AAG CTT gaa atc aaa cgg GCC TCC ACA CAG AGC CCA (SEQ ID NO: 27)

and

gcgc ctcgag TCA TTT ACC GGG ATT TAC AGA (SEQ ID NO: 28)

This is then restricted with those enzymes and inserted into pBSII/Leader/ScFv restricted with the same enzymes, creating pBSII/Leader/ScFv/HE1.

Page 76, line 1:

Using cDNA derived by RT of RNA isolated from a cell line such the 293 human kidney line (ATCC: CRL1573), the DNA is amplified by PCR using oligonucleotides containing a *Spe I* restriction enzyme site at the N-terminus and a stop codon and a *Not I* site at the C-terminus

GG ACT AGT AAT AGT GAC TCT GAA TGT CCC (SEQ ID NO: 29)

And

ATT AGC GGC CGC TTA GCG CAG TTC CCA CCA CTT C (SEQ ID NO: 30)

The resulting product is digested with those enzymes and ligated to pBS/B7 Link which has been restricted with the same enzymes creating pBS/B7 Link EGF. The B7 Link EGF cassette is then excised with *Eco RI* and *Not I* and inserted into a derivative of pHIT111 (Soneoka *et al*, 1995, Nucl Acid Res 23; 628) which no longer carries the *LacZ* gene.

Page 82, line 31:

For B7-5T4 scFv the primers are as follows:-

Primer 1. B7-Sbf

ATCGCCTGCAGGCC***ACCATGG***CCTTGCAATTGTCAG (SEQ ID NO: 31)

Sbf I site = underlined

Kozak sequence = bold and italics with the ATG start codon underlined.

Primer 2. 5T4sc-RIGCGCGAATTCTTACCGTTTGATTTCAGCTTGGT (SEQ ID NO: 32)

Eco RI site = underlined

TAA stop codon = bold and italics

The resultant product is then cloned into pONY 8.1 SM to produce the fusion protein construct shown in Figure 19a.

For L-5T4 scFv the primers are as follows:-**Primer 1. L-Sbf**ATCGCCTGCAGG***CCACCATG***GGATGGAGCTGTAT (SEQ ID NO: 33)

Sbf I site = underlined

Kozak sequence = bold and italics, with the ATG start codon underlined.

Primer 2. 5T4sc-RIGCGCGAATTCTTACCGTTTGATTTCAGCTTGGT (SEQ ID NO: 34)

Eco RI site = underlined

TAA stop codon = bold and italics

The resultant product is then cloned into pONY 8.1 SM to produce the construct shown in Figure 19b.